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Synthesis and micellization of amphiphilic biodegradable methoxypolyethylene glycol/poly(D,L-lactide)/polyphosphate block copolymer

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ABSTRACT

A new amphiphilic biodegradable methoxypolyethylene glycol/poly(D,L-lactide)/poly(ethyl ethylene phosphate) (MPEG-*b*-PLA-*b*-PEEP) block copolymer was synthesized by ring-opening polymerization of ethyl ethylene phosphate (EEP) with methoxypolyethylene glycol/poly(D,L-lactide) (MPEG-*b*-PLA) as a macroinitiator, which was prepared by ring-opening polymerization of D,L-lactide (LA) initiated by methoxypolyethylene glycol (MPEG) using stannous octoate as catalyst. The structures of the block copolymers were confirmed by IR, ^{1}H NMR and GPC analysis. Fluorescence measurements were applied to determine the critical micelle concentration (CMC) of the copolymer micelle solutions. The diameter and the distribution of micelles were characterized by dynamic light scattering (DLS) and the shape was perceived using transmission electron microscopy (TEM). The results prove that the copolymers can self-assemble into nano-micelles in aqueous solutions. The CMC of the copolymer solutions increased and the size of the micelles reduced with increasing of the proportion of PEEP segments. TEM images demonstrate that all micelles are spherical.

1. Introduction

Amphiphilic block copolymers with the ability to self-assemble have extensively investigated for biomedical applications [1]. They can be used as controlled release agents of drugs, proteins, etc. because their biodegradability and amphiphilicity [2,3]. Lipophilic drug molecules can be incorporated into the hydrophobic core of polymeric micelles by physical entrapment, while the hydrophilic shell composed of flexible polymers provides steric protection [4].

Polylactide (PLA), which is most commonly synthesized by the ring opening polymerization of lactide [5,6], is a biodegradable polyester having good biocompatible properties. As a consequence, it has been utilized as a valuable bio-absorbent in medical and pharmaceutical fields [7–11]. Because PLA is hydrophobic and degrade very slowly by simple hydrolysis under the human body conditions, hydrophilic segment was usually introduced into the PLA polymer chain.

Recently, polyphosphoester (PPE) becomes of interest for biological and pharmaceutical applications because of their biocompatibility and structural similarities to natural occurring nucleic and teichoic acids [12]. PPE represents a class of biodegradable polymers with repeated phosphoester attachments in the backbone, which degrades under the physiological conditions via hydrolysis or enzymatic cleavage of the phosphoester bonds [13–17]. The degradation rates and other physico-chemical properties of these polymers are controllable by the chemical structure in the backbone and side chain [18]. More recently, PPE has received substantial interest in a wide range of applications from drug and gene delivery to tissue engineering [19–22]. Furhtermore, poly(ethyl ethylene phosphate) (PEEP), a typical hydrophilic polyphosphoester, also received considerable attention in biomedical applications due to its biodegradability and good biocompatibility. If the PLA chain is combined with PEEP to prepare an amphiphilic biodegradable polymer, its hydrophilicity and biodegradability can be regulated, and thus its applications may extend widely.

In this paper, a new amphiphilic biodegradable methoxypolyethylene glycol/poly(D,L-lactide)/poly(ethyl ethylene phosphate) (MPEG-*b*-PLA-*b*-PEEP) block copolymer was synthesized by ring-opening polymerization of cyclic ethyl ethylene phosphate (EEP) with methoxypolyethylene glycol/poly(D,L-lactide) (MPEG-*b*-PLA) as a macroinitiator. Its structure was characterized and the self-assembling properties of the copolymer were investigated.

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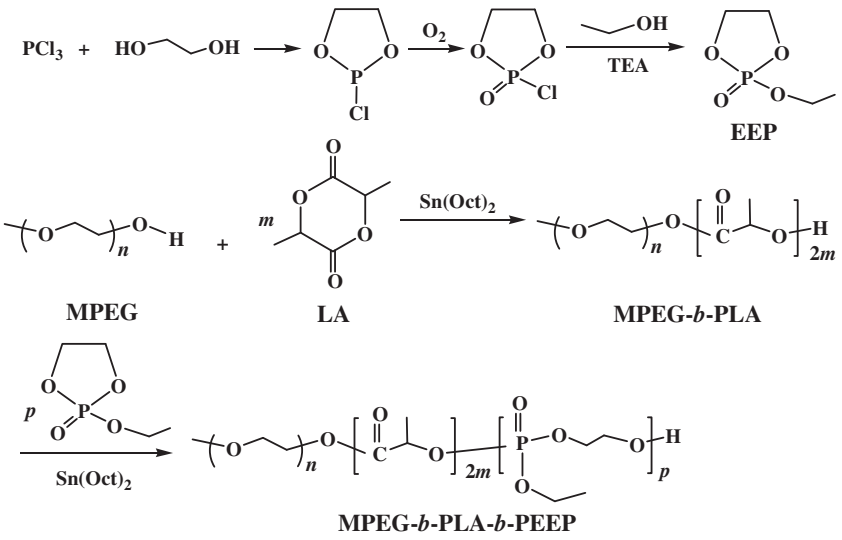

Scheme 1. The synthesis of MPEG-*b*-PLA-*b*-PEEP block copolymer.

2. Experimental

2.1. Materials

D,L-lactide (LA) was purchased from Daigang Biotechnology Co., Ltd. (Shandong, China) and recrystallized from dry ethyl acetate prior to use. Methoxypolyethylene glycol (MPEG, M_n = 5000) (Fluka Chemical Reagent Co., Ltd.) was distilled with toluene (azeotropic) before use. Stannous octoate ($Sn(Oct)_2$) was purchased from Sigma–Aldrich Chemical Reagent Co., Ltd. Ethylene glycol (analytical grade), triethylamine (analytical grade), ethanol (analytical grade) and CH_2Cl_2 (analytical grade) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), dried and distilled prior to use. Phosphorus trichloride (analytical grade) was purchased from Guangfu Fine Chemical Research Institute (Tianjin China). Toluene (analytical grade) was purchased from Baishi Chemical Reagent Co., Ltd. (Tianjin, China), dried and distilled before use. Other chemicals are all analytical reagents made in China and used without further purification.

2.2. Preparation of ethyl ethylene phosphate (EEP)Scheme 1

Ethyl ethylene phosphate (EEP) was prepared according to the procedure [23]. Briefly, to a stirred mixture of 274.66 g (2 mol) of phosphorus trichloride and 250 mL of dry CH_2Cl_2, 124.14 g (2 mol) of ethylene glycol was added drop wise. After complete addition of ethylene glycol, the solution was stirred at room temperature for another 0.5 h and CH_2Cl_2 was evaporated under vacuum. The residue was distilled under reduced pressure to give 123.2 g of 2-chloro-1,3,2-dioxaphospholane (yield: 49%, b.p. 42–45 °C/1600 Pa).

The oxidation of 123.2 g 2-chloro-1,3,2-dioxaphospholane was carried out by bubbling O_2 through the toluene solution at 40 °C for 48 h. After removal of toluene, the residue was distilled under reduced pressure to give 77.9 g colorless liquid of 2-chloro-2-oxo-1,3,2-dioxaphospholane(yield: 56%, b.p. 88–90 °C/107 Pa).

To a stirred and cooled mixture (−5 °C) containing 77.9 g (0.55 mol) of 2-chloro-2-oxo-1,3,2-dioxaphospholane and 250 mL dry toluene, a mixture of 25.3 g (0.55 mol) dry ethanol and 61.6 g (0.605 mol) dry triethylamine was added drop wise, and then the resulting mixture was stirred at room temperature for another 2 h. Thereafter, the triethylamine hydrochloride was filtered off and the filtrate was concentrated. The residue was distilled under reduced pressure to give 90.5 g colorless liquid of ethyl ethylene phosphate (EEP) (yield: 61%, 95–97 °C/107 Pa).

2.3. Synthesis of methoxypolyethylene glycol/poly(D,L-lactide)(MPEG-b-PLA) macroinitiator

MPEG-*b*-PLA was prepared according the literature [24]. In brief, 0.505 g (0.1 mmol) of MPEG, 2.954 g (20.5 mmol) of LA and 0.009 g (0.02 mmol) of $Sn(Oct)_2$ were transferred to a 10 mL ampoule and after six cycles of evacuation–purging with purified nitrogen the ampoule was sealed. The polymerization reaction was performed in an oil bath at 140 °C and terminated after 48 h. After being cooled to room temperature, the resulting polymer was dissolved in 15 mL anhydrous CH_2Cl_2 and precipitated in 150 mL ethyl ether twice. The precipitate was dried under reduced pressure at 35 °C for 24 h giving the MPEG-*b*-PLA macroinitiator as pale yellow solid, yield: 40% (Scheme 1). The copolymers were characterized by IR, ^{1}H NMR and GPC analysis. M_n is 22,424 (determined by ^{1}H NMR). The copolymers gave a broad absorption in the 3200–3600 cm^{-1} region due to terminal hydroxyl groups. Furthermore, the peak at 1757 cm^{-1} was assigned to the stretch vibration $\nu_{C=O}$ from the PLA segment, the typical absorption of the lactide monomer at 935 cm^{-1} had completely disappeared (Fig. 1A). ^{1}H NMR ($CDCl_3$, δppm): 1.56 (3H, —CHC**H**$_3$ of PLA), 3.40 (3H,

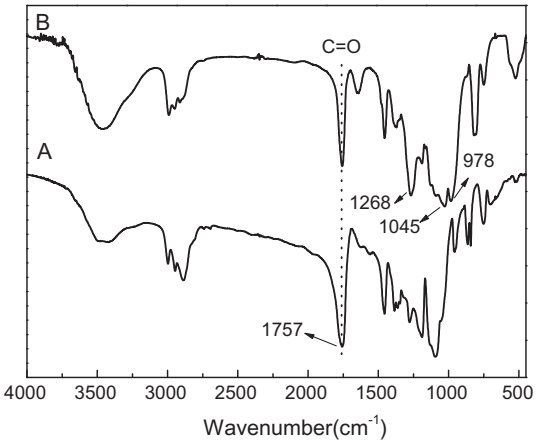

Fig. 1. IR spectra of MPEG-*b*-PLA (A) and MPEG-*b*-PLA-*b*-PEEP**2** (B) block copolymers.

Table 1
Related data on block copolymers.

Sample	MPEG-*b*-PLA/EEP[a]	$W_{PEEP}/W_{MPEG\text{-}b\text{-}PLA}$[b]	M_n[b]	M_n[c]	M_w/M_n	CMC × 10^3 (mg/mL)	Size (nm)[d]
MPEG-*b*-PLA	/	/	22,424	19,907	1.41	/	/
MPEG-*b*-PLA-*b*-PEEP**1**	1/100	11/89	25,216	20,504	1.43	1.0	116
MPEG-*b*-PLA-*b*-PEEP**2**	1/350	54/46	48,912	29,296	1.22	2.5	105
MPEG-*b*-PLA-*b*-PEEP**3**	1/450	61/39	56,936	36,889	1.08	3.2	97

[a] Molar ratio of MPEG-*b*-PLA to EEP.
[b] Determined by ^{1}H NMR in $CDCl_3$ solution.
[c] Determined by GPC in THF at 30 °C.
[d] Determined by dynamic light scattering in aqueous solution at 25 °C.

C**H**$_3$O— of MPEG), 3.64 (4H, —OC**H**$_2$C**H**$_2$— of MPEG), 5.16 (1H, —C**H**CH$_3$ of PLA). From GPC analysis it followed that the block copolymers own a unimodal molecular weight distribution (Fig. 3A). This indicated that the copolymerization was completed successfully and no homo-polymerization occurred during the reaction.

2.4. Synthesis of methoxypolyethylene glycol/poly(D,L-lactide)/poly(ethyl ethylene phosphate) (MPEG-b-PLA-b-PEEP) block copolymers (Scheme 1)

The necessary amounts of MPEG-*b*-PLA, EEP and $Sn(Oct)_2$ were added to a 10 mL ampoule and after six cycles of evacuation–purging with purified nitrogen the ampoule was sealed. The polymerization reaction was performed in an oil bath at 90 °C and terminated after 24 h. After being cooled to room temperature, the product was dissolved in 15 mL THF and precipitated in 150 mL ethyl ether twice. The precipitate was dried under reduced pressure at 35 °C for 24 h to give the desired MPEG-*b*-PLA-*b*-PEEP block copolymer as white solid. The yield was approximately 40%. Different molar ratios of the feeding EEP to MPEG-*b*-PLA resulted in the corresponding copolymers with various compositions are listed in Table 1.

2.5. Characterization of the block copolymers

The IR spectra were collected by a Perkin–Elmer FT-IR spectrometer using KBr disks. ^{1}H NMR spectra were recorded on a Varian Mercury-300 NMR spectrometer at room temperature, using $CDCl_3$ as solvent. Chemical shifts (δ) were given in ppm using tetramethylsilane (TMS) as an internal reference. The gel permeation chromatography (GPC) was conducted using a Waters 1515 GPC instrument equipped with a HT4 column (effective molecular-weight range: 5000–600,000) and a 2414 differential refractive index detector. THF was applied as eluent at a flow rate of 1.0 mL/min at 30 °C. The molecular weights were calibrated utilizing polystyrene standards.

2.6. Preparation of micelles

The micelles were prepared applying a solvent displacement method with a tetrahydrofuran/water (THF/H_2O) system [25]. A copolymer (50 mg) was first dissolved in 2.5 mL of THF, thereafter the copolymer solution was slowly added into 10 mL of ultrapurified water (Aquaplus 18.2 MΩ). The THF was removed using a rotary evaporator at 25 °C for 2 h. The obtained solution was transferred into a 25 mL volumetric flask, followed by dilution to the calibration mark with ultrapurified water to obtain 2 mg/mL micelles.

2.7. Micelle characterization

The critical micelle concentrations (CMC) of the copolymers were determined by fluorescence measurements using pyrene as a probe. A pyrene solution (in acetone) was added into a series of volumetric flasks in such an amount that the final concentration of pyrene in each solution was 5.93×10^{-7} mol/L, thereafter the acetone was removed completely. The polymer solution was added into the volumetric flasks and diluted till the calibration mark using ultrapurified water to obtain the desired copolymer concentrations ranging from 1.0×10^{-6} mg/mL to 1.0 mg/mL. The samples were heated at 50 °C for 2 h, and stored at room temperature overnight to equilibrate the pyrene and micelles. Steady-state fluorescence excitation spectra were recorded on a Varian Cary Eclipse fluorescence spectrophotometer at 390 nm emission wavelength and 2.5 nm slit width. The scan rate was 120 nm/min. The size distribution of micelles was determined by dynamic light scattering (DLS) using a Malvern Nano ZS instrument at 25 °C. The morphology of the micelles was investigated by transmission electron microscopy (TEM), carried out on a Hitachi H-7650 electron microscope, operating at an accelerating voltage of 80 kV. Specimens were prepared by transferring a drop of the micelle solution onto a 200 mesh copper grid coated with carbon and allowing the sample to dry in air before measurements.

3. Results and discussion

3.1. Synthesis of MPEG-b-PLA-b-PEEP block copolymer

Stannous octoate, one of the most widely used initiators for cyclic esters polymerization, has been reported recently to induce polymerization of cyclic ε-caprolactone (ε-CL), lactide (LA), and phosphoester (PPE) by formation of stannous alcoholate active centers with ROH or RNH_2 as co-initiator [15,26,27]. Because MPEG-*b*-PLA contains hydroxyl groups, it can initiate ring-opening polymerization of cyclic EEP to generate MPEG-*b*-PLA-*b*-PEEP block copolymers. A series of the block copolymers with various molecular weights were synthesized and the results are summarized in Table 1. It is found that the total molecular weight of the

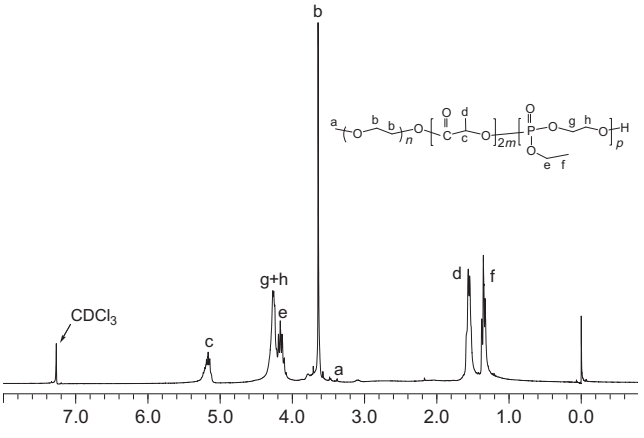

Fig. 2. ^{1}H NMR spectrum of MPEG-*b*-PLA-*b*-PEEP**2** block copolymer in $CDCl_3$.

copolymers increased with the molar ratio of monomer EEP to the initiators.

The IR spectrum of MPEG-*b*-PLA-*b*-PEEP**2** is depicted in Fig. 1B. Compared to MPEG-*b*-PLA (Fig. 1A), the spectrum of the copolymer clearly shows the most important vibration bands, especially the vibrations of P═O appearing at 1268 cm^{-1}, P—O—C at 1045 and 978 cm^{-1}.

The ^1H NMR spectrum of the MPEG-*b*-PLA-*b*-PEEP**2** triblock copolymer is shown in Fig. 2. The peaks at 3.40 and 3.64 ppm are assigned to protons a and b in the MPEG segment, respectively. The peaks at 1.55 and 5.16 ppm are assigned to protons d and c in the PLA segment, respectively. The peaks at 1.35, 4.16 and 4.25 ppm are assigned to protons f, e and g + h in the PEEP segment, respectively. No additional peaks were detected in the spectrum, implying the block copolymer prepared.

The GPC chromatograms of the block copolymers are depicted in Fig. 3. The three copolymers show a unimodal molecular weight distribution, indicating that the copolymerization was completed successfully and that no homo-polymers were generated. GPC data of the copolymers are listed in Table 1.

3.2. Formation of micelles

Pyrene has been widely used as a probe to monitor the association and micellization of macromolecules in solution because its photophysical character changes with the variation of the existing environment [28]. The micellar structures of MPEG-*b*-PLA-*b*-PEEP were confirmed by fluorescence spectroscopy using pyrene as a probe. The fluorescence excitation spectra of pyrene in the presence of MPEG-*b*-PLA-*b*-PEEP**2** at various concentrations are shown in Fig. 4. A red shift from 334 nm to 336 nm is observed with increasing concentration of MPEG-*b*-PLA-*b*-PEEP**2**, indicating that micellization took place for the MPEG-*b*-PLA-*b*-PEEP copolymer. Such results can be attributed to the transfer of pyrene molecules from water to a hydrophobic environment within the micelle cores.

In addition, the onset of micellization and the critical micelle concentrations (CMC) can be obtained from the excitation spectra

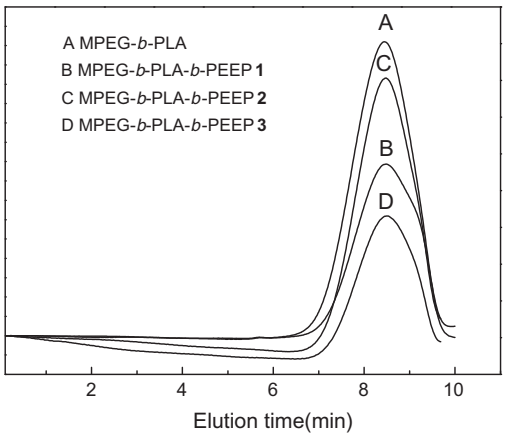

Fig. 3. GPC chromatograms of the block copolymers.

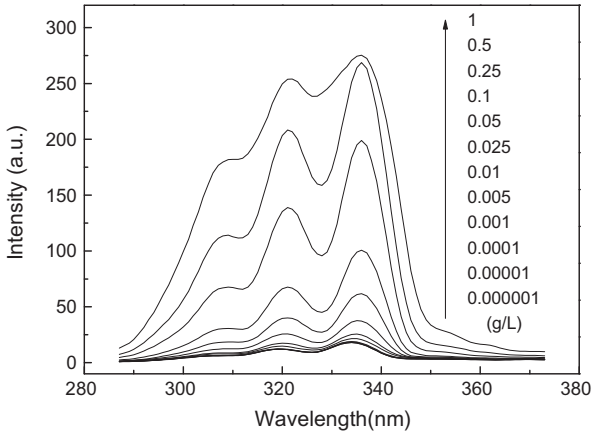

Fig. 4. Excitation spectra of pyrene as a function of MPEG-*b*-PLA-*b*-PEEP**2** concentration in water.

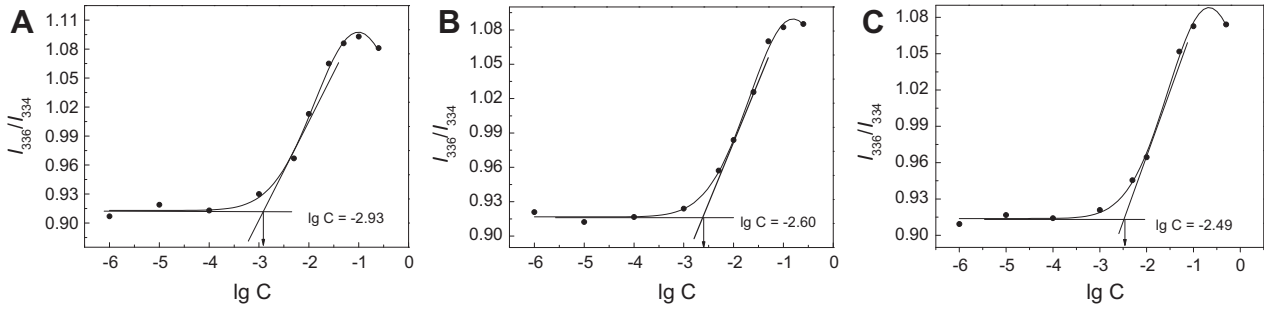

Fig. 5. Plots of I_{336}/I_{334} versus logarithm of block copolymers concentrations.

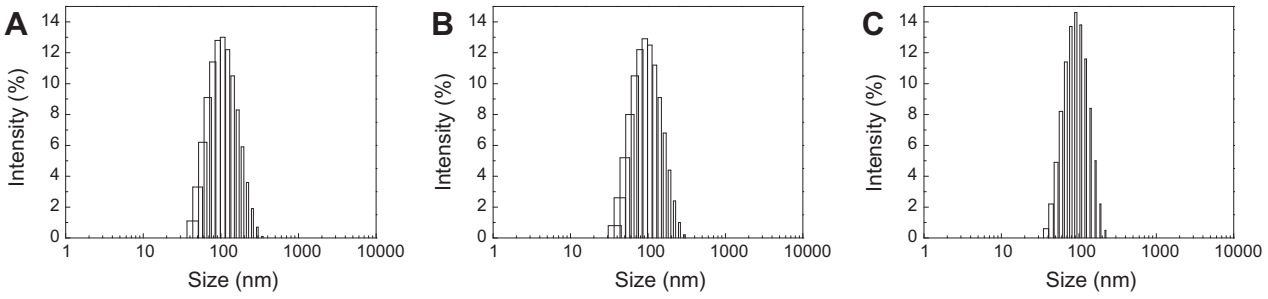

Fig. 6. The size distributions of MPEG-*b*-PLA-*b*-PEEP**1** (A), MPEG-*b*-PLA-*b*-PEEP**2** (B) and MPEG-*b*-PLA-*b*-PEEP**3** (C) block copolymer micelles in aqueous solution measured by DLS.

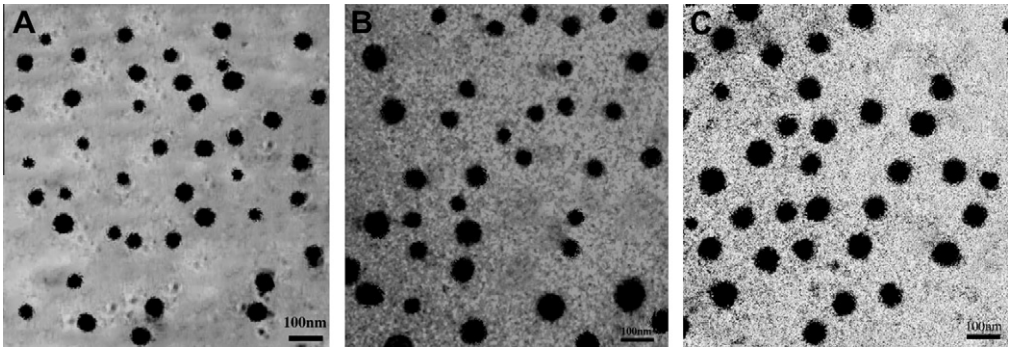

Fig. 7. TEM images of MPEG-*b*-PLA-*b*-PEEP**1** (A), MPEG-*b*-PLA-*b*-PEEP**2** (B) and MPEG-*b*-PLA-*b*-PEEP**3** (C) block copolymer micelles.

[29]. For the copolymer MPEG-*b*-PLA-*b*-PEEP**2**, 334 nm and 336 nm were chosen as the peaks wavelength of the (0, 0) band in the pyrene excitation spectra in the aqueous phase and in the entirely hydrophobic core of polymeric micelle, respectively. The plots of the pyrene fluorescence intensity ratios (I_{336}/I_{334}) versus the logarithm of copolymer concentrations are depicted in Fig. 5. Below a certain concentration, I_{336}/I_{334} is constant while above this concentration, I_{336}/I_{334} increases with rising lgC and finally reaches a plateau. From these plots, the critical micelle concentrations (CMC) were obtained from the intersection of two straight lines: the base line and the rapidly rising I_{336}/I_{334} line. The CMC values of the MPEG-*b*-PLA-*b*-PEEP block copolymers are listed in Table 1. The CMC values are in the magnitude of 10^{-3} mg/mL and diminish with the enlargement of the PEEP segment, indicative of the amphiphilic nature of MPEG-*b*-PLA-*b*-PEEP block copolymers.

3.3. Size and size distribution of MPEG-b-PLA-b-PEEP micelles

The size and size distribution of the micelles were measured using DLS. As shown in Fig. 6, the mean diameter of the micelles, formed by MPEG-*b*-PLA-*b*-PEEP**1**-**3** are about 116, 105 and 97 nm, respectively. The size of the micelle reduces with the extension of the PEEP segment, so the size of the copolymer micelles can be adjusted by modification of the size of the PEEP segment from the copolymer.

3.4. Morphology of MPEG-b-PLA-b-PEEP micelles

The morphology of the micelles was examined by TEM. Fig. 7 presents the TEM images of the micelles. They are all spherical and the micelles sizes formed by MPEG-*b*-PLA-*b*-PEEP**1**-**3** are about 89, 80 and 75 nm, while the standard deviations are 30.3585, 30.5824 and 53.5138 respectively. The diameter data are smaller than that determined by DLS, since the micelle diameter determined by DLS represents their hydrodynamics diameter while the diameter obtained by TEM is related to the collapsed micelles after water evaporation.

4. Conclusions

A new amphiphilic biodegradable MPEG-*b*-PLA-*b*-PEEP block copolymer was synthesized by ring-opening polymerization of EEP using MPEG-*b*-PLA as a macroinitiator. Characterizations using IR, ^{1}H NMR and GPC confirmed the designed structure. Via self-assembling, nano-micelles in water were generated. The CMC value, measured by fluorescence spectroscopy, is in the range of 10^{-3} mg/mL and increases with the enlargement of the PEEP segment. The average micelle size determined by DLS reduced with the extension of the PEEP segment and the TEM images demonstrated that all micelles are spherical. The latter property would be extremely applicable in drug delivery systems and other biomedical fields.

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